

# Inflammation and neutrophil immunosenescence in health and disease

Drew, William; Wilson, Daisy; Sapey, Elizabeth

DOI:

[10.1016/j.exger.2017.12.020](https://doi.org/10.1016/j.exger.2017.12.020)

[10.1016/j.exger.2017.12.020](https://doi.org/10.1016/j.exger.2017.12.020)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Drew, W, Wilson, D & Sapey, E 2018, 'Inflammation and neutrophil immunosenescence in health and disease: Targeted treatments to improve clinical outcomes in the elderly', *Experimental gerontology*, vol. 105, pp. 70-77. <https://doi.org/10.1016/j.exger.2017.12.020>, <https://doi.org/10.1016/j.exger.2017.12.020>

[Link to publication on Research at Birmingham portal](#)

## **Publisher Rights Statement:**

Checked for eligibility: 08/01/2018

## **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

**Inflammation and neutrophil immunosenescence in health and disease: Targeted treatments to improve clinical outcomes in the elderly.**

William Drew<sup>1</sup>, Daisy V Wilson<sup>1</sup>, Elizabeth Sapey<sup>1,2</sup>

1 = Institute of inflammation and Ageing, University of Birmingham, Birmingham, West Midlands, UK

2 = Senior and corresponding author.

Institute of inflammation and Ageing, University of Birmingham, Birmingham, West Midlands, UK, B15 2GW

[e.sapey@bham.ac.uk](mailto:e.sapey@bham.ac.uk)

Telephone: +44 121 627 2000

Key words:

Neutrophils; Inflammation; Pneumonia; Sepsis; Statins; Phosphoinositide 3 kinase; Epigenetic.

Drs Sapey and Wilson are supported by the Medical Research Council. The authors have no conflicts of interest in relation to this submitted review.

## **Abstract**

Despite increasing longevity, many old people are not in good health. There has been an increase in the prevalence of age-associated multi-morbidity (two or more chronic conditions in the same person). Also, severe infections, such as pneumonia, remain significant causes of mortality and morbidity in this aging group. Many chronic health conditions share risk factors such as increasing age, smoking, a sedentary life style and being part of a lower socioeconomic group. However, despite this, multi-morbidities often co-occur more commonly than would be predicted. This has led to the hypothesis that they share common underlying mechanisms. This is an important concept, for if it were true, treatments could be devised which target these common pathways and improve a number of age-associated health conditions.

Many chronic illnesses associated with multi-morbidity and severe infections are characterised by an abnormal and sustained inflammatory response, with neutrophils being key effector cells in the pathological process. Studies have described aberrant neutrophil functions across these conditions, and some have highlighted potential mechanisms for altered cell behaviours which appear shared across disease states. It has been suggested that altered functions may represent neutrophil “senescence”. This review considers how and why neutrophil functions change as the cell ages, and how and why neutrophil functions change as the host ages in health and disease and discusses whether neutrophil functions could be targeted to improve health outcomes in older adults.

## Introduction

Our population is aging, but longevity is not always associated with good health and an increasing number of our older population are burdened with frailty, ill-health and functional limitation<sup>1</sup>. There has been a substantial rise in the prevalence of chronic, non-communicable diseases associated with age<sup>2</sup>. For example, the prevalence of cardiovascular disease (including hypertension, coronary heart disease and heart failure) increases from 40% in people aged 40-59 years of age<sup>3</sup> to 70% in people aged 70 years of age<sup>4</sup>; chronic obstructive pulmonary disease (COPD) increases from 8% in adults aged 50 to 20% of adults aged 70<sup>5</sup>. Furthermore, it is increasingly common for an older person to suffer with a number of medical conditions. Multi-morbidity (defined as two or more chronic diseases in one person<sup>6</sup>) affects approximately 65% of over 60 year olds, of which 80% live with disabilities<sup>6</sup> and multi-morbidity accounts for 60% of global deaths<sup>7</sup>. The World Health Organisation has recognized this burden of ill-health (increased lifespan with multi-morbidity) as a major challenge to be faced by global health care systems<sup>8</sup>, and in 2016, the first multi-morbidity care guidelines were published<sup>9</sup>, although the evidence base for multi-morbid health care pathways is limited.

The most common chronic non-communicable diseases are associated with inflammation, for example, COPD<sup>10</sup>, type 2 diabetes<sup>11</sup>, osteoporosis<sup>12</sup> and dementia<sup>13</sup> and these conditions often co-occur. Recent studies have demonstrated patterns of disease clustering<sup>14</sup>, with links seen between cardiovascular disease risk factors and conditions, metabolic conditions, and pain, musculoskeletal and psychological conditions<sup>15</sup>. Many of these conditions share risk factors of age, cigarette smoking, lower socioeconomic group, and sedentary lifestyle but the odds ratio of them occurring together are greater than would be predicted once these common shared risk factors are taken into account<sup>3,16</sup>.

As well as chronic illness, there is a significant burden of infection related mortality and morbidity in our aging population. Pneumonia (a severe lung infection defined by symptoms of a lower respiratory tract infection and new consolidative changes on a chest radiograph<sup>17</sup>) remains the commonest form of infectious death in the developed world, the fifth leading cause of death worldwide. Pneumonia is diagnosed in approximately 10.6 per 1000 person years<sup>18</sup> but 75 per 1000 person years in adults aged over 70<sup>19</sup>. Deaths are

highest in old patients, with mortality rates not improved over the last decade<sup>20,21</sup>. Far from being an acute infection, many who survive the initial episode are frailer and require more social support than they did prior to admission<sup>22,23</sup>. Recovery from the primary infection is also associated with an increased risk of secondary infections, and the outcomes from these events is even less certain<sup>24</sup>.

Acute bacterial infections such as pneumonia require a functional immune system, and in particular, a coordinated and controlled innate immune response to clear infection without causing excessive host tissue damage; with the neutrophil being a key effector cell. These cells have been implicated also in the pathogenesis of many of the co-morbidities present in old age. For example, neutrophils are associated with lung tissue destruction in COPD (as reviewed in <sup>25</sup>) but are also implicated in myocardial infarction<sup>26</sup>, and type 2 diabetes <sup>27</sup> and in more general features of ill health in old age, including frailty<sup>28</sup>.

### **Classical neutrophil functions**

Neutrophils comprise 70% of circulating white blood cells, but have a short lifespan (approximately a half-life of eight hours) necessitating a high daily production rate of  $1-2 \times 10^{11}$  cells/day in health<sup>29-31</sup>. Defined as granulocytes and phagocytes, neutrophils contain a specialized antimicrobial granule system and ingest target particles such as bacteria<sup>29,32</sup>. During maturation, neutrophils develop their characteristic granules, which are traditionally divided into three sub-types; azurophilic (primary), specific (secondary) and gelatinase (tertiary). Azurophilic granules contain the neutral serine proteinases, (neutrophil elastase, proteinase 3, and cathepsin G) myeloperoxidase (MPO) and other antimicrobial proteins (e.g.  $\alpha$ -defensins)<sup>32,33</sup>. Specific (secondary) granules are also predominantly bactericidal and contain products such as lactoferrin<sup>32,34,35</sup>. Gelatinase (tertiary) granules contain metalloproteases (MMPs) such as gelatinase which digest extracellular matrix and aid neutrophil migration<sup>29,36</sup>. Neutrophils are also able to generate reactive oxygen species (ROS) by NADPH oxidase, an enzyme which is only activated when its cytosol-based and membrane-based component parts bind, a process stimulated by pathogenic and host-derived inflammatory signals<sup>37</sup>. Degranulation describes mobilization of granules and fusion with the neutrophil membrane, causing release of contents outside the cell or into a phagosomal vacuole<sup>32</sup>. When released, granules are both anti-microbial

and cytotoxic, and have the potential for significant tissue damage, particularly when they are first released from the neutrophil, as here inhibitors of granule contents (such as Alpha 1 Antitrypsin which inhibits neutrophil elastase) are insufficient in concentration to prevent local protein degradation<sup>38</sup>.

After release from the bone marrow, neutrophils circulate in systemic blood, displaying high levels of physical plasticity, allowing them to move through capillary networks with diameters half that of the quiescent neutrophil by elongating their cell shape, without significant delay in transit times<sup>39,40</sup>. Towards the end of their short half-life, neutrophils develop an ageing phenotype with increased surface expression of CXCR4<sup>41</sup> which is thought to facilitate clearance by apoptosis and subsequent efferocytosis (clearance of neutrophils by phagocytosis) by stromal macrophages.

During an inflammatory or infectious challenge, neutrophils migrate with great accuracy through tissue to sites of infection and inflammation, where they phagocytose bacteria and cell debris. Direction sensing is achieved via occupancy of G-protein coupled receptors (GPCRs) on the surface of the neutrophil, as soluble chemo-attractants (such as Interleukin-8 (CXCL8) or bacterial N-formyl-methionyl-leucylphenylalanine (fMLP)) form gradients, which neutrophils sense by the binding of ligands to receptors at the leading edge of the cell<sup>42</sup>. When chemokine's bind their cognate receptors, dissociation of heterotrimeric G proteins activate phosphatidylinositol-3 kinase (PI3K), an enzyme which has been implicated in the regulation of migration<sup>43</sup>, phagocytosis<sup>44</sup> and azurophil degranulation<sup>45</sup>. PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) to phosphatidylinositol 3,4,5-trisphosphate (PIP<sub>3</sub>). In health, fluctuations in PIP<sub>3</sub> are associated with the localized formation of pseudopodia (temporary cytoplasm-filled projections from the neutrophil cell membrane), which steer the cytoskeletal rearrangement required to orient the neutrophil to chemo-attractant cues<sup>46</sup> and are required for migration and opsonophagocytosis. There are a number of PI3K classes and isoforms, but Class 1 delta and gamma isoforms are highly expressed in neutrophils, and thought central to accurate migration. Downstream of PI3K, the small GTPases (RhoA, Rac and CDC42) organize cell polarity, motility and phagocytosis with Rac and CDC42 localizing at the leading edge of the cell<sup>47</sup> and RhoA and ROCK driving propulsion at the rear of the cell<sup>48</sup>. Migration through the dense extracellular matrix is

facilitated by the sequential release of proteinases and reactive oxygen species around the neutrophil<sup>49</sup>, degrading a path to assist passage. Thus, the process of migration can be associated with tissue damage.

Phagocytosis is an active, receptor mediated process<sup>50</sup>. In unopsonized phagocytosis, interactions between neutrophilic pattern recognition receptors (PRRs) (such as Toll Like Receptors<sup>51</sup>) and surface-expressed pathogen-associated molecular patterns (PAMPs) support slow bacteria envelopment<sup>29</sup>. Opsonized phagocytosis is a much more dynamic process, where neutrophils internalize bacteria through their opsonin receptors, Fc receptors and a sub-group of  $\beta$ 2-integrins, which bind complement<sup>52</sup>. In the presence of overwhelming infection or during ingestion of large particles, neutrophils display “frustrated phagocytosis” where they degranulate and release proteinases and reactive oxygen species around the semi-internalized particle into the extracellular matrix<sup>53</sup>, causing localized tissue damage. More recently, Neutrophil Extracellular Traps (NETs) have been described, which are large, complex, fibrous structures formed from nuclear chromatin and granular proteins<sup>54</sup> which are extruded from neutrophils to ensnare bacteria, bringing them into close association with antimicrobial proteins. These appear to represent a neutrophil’s final response to overwhelming infection and inflammation but are also associated with significant local tissue damage<sup>55</sup>, caused in part by the effects of proteinases which are more resistant to anti-proteinase inhibition when membrane bound<sup>56</sup>. Figure 1 provides an overview of these functions.

Neutrophils are thought to exist in three states; quiescent, activated or primed<sup>57</sup>. An activated cell is able to degranulate, produce significant quantities of ROS and release NETs, all of which could harm host tissue. Priming appears to be an intermediate and protective step prior to full activation, from which a cell can step up, and become activated, or step down, and become quiescent, providing a natural break before bacteriocidal and cytotoxic products are released<sup>57</sup>.

### **The aging neutrophil**

Most of our understanding of the lifespan of a neutrophil is based on murine models.

Circulating neutrophil numbers do not remain constant throughout the day and neutrophils are released and cleared in waves. C-X-C chemokine receptor type 2 (CXCR2) and CXCR4 regulate neutrophil trafficking from the bone marrow. The interaction between CXCR4 and the stromal cell-derived factor 1 (SDF1), also known as C-X-C motif chemokine 12 (CXCL12) retains neutrophils within the marrow environment, while CXCR2 regulates neutrophil mobilization through antagonism of CXCR4 signaling<sup>58</sup>. Fresh, young neutrophils are released from the bone marrow during the resting phase of an animal's life (night, for most humans), with absolute neutrophil numbers peaking at this time.

Like all cells in the body, neutrophils age and are susceptible to damage. Aged neutrophils are cleared at the end of the resting phase of the day<sup>59</sup>. These oscillations are thought to provide a pool of protective neutrophils at the time when exposure to pathogenic microorganisms is believed to be more likely. Potential damaging triggers are thought to include exposure to external signals (including the microbiota<sup>60</sup> and free radicals), however, unlike monocytes or eosinophils, neutrophils are thought to have little capacity for DNA repair<sup>61</sup>. Cellular aging leads to specific changes in the appearance, surface expression and function of neutrophils (reviewed elegantly in <sup>62</sup>).

In brief, freshly released neutrophils are thought to express high levels of L-selectin (CD62L) on their surface but low levels of CXCR4. Exposure to low grade inflammation increases CD11b/c/ ICAM1 and CD49d expression, which in turn promotes migration through the endothelium. Following migration to tissues, cells with low levels of CD47 are more likely to be phagocytosed by tissue macrophages (a process termed efferocytosis). As the cells age, they express more CXCR4, which is the receptor for SDF-1 and consequently, aged neutrophils migrate to areas of high SDF-1 production, including the bone marrow, where they can be cleared following apoptosis<sup>63</sup>.

Aging does not always lead to neutrophil clearance however, and during challenges such as sepsis, the redirection of aged neutrophils to bone marrow appears to be suspended. In a recent study, Toll like receptors (TLR-4 in particular) enabled the recruitment of aged neutrophils to an inflammatory focus where the aged cells had an increased capacity to phagocytose gram-negative and gram-positive bacteria without a corresponding rise in



respiratory burst or cytokine production<sup>64</sup>. In contrast, in a different murine model by Zhang et al reported that aged neutrophils (in response to bacterial products) could produce more NETs and ROS<sup>60</sup>. It remains unclear, therefore whether retaining an aged population of neutrophils is beneficial or potentially damaging, and it is also unclear whether these murine responses are present in humans.

In reproducing cells, senescence is distinct from being old. Senescence is the process by which cells irreversibly stop dividing and enter a state of permanent growth arrest without undergoing cell death, caused by telomere erosion, DNA damage or the aberrant activation of oncogenes. Neutrophils never divide, and therefore it is unclear when, or if, they become senescent, and how one might recognize this state. In other cells, senescence is recognized as a negative and pro-inflammatory condition. Senescent cells secrete various cytokines, chemokines, matrix remodelling proteases and growth factors, a phenotype collectively referred to as the senescence-associated secretory phenotype (SASP)<sup>65</sup>. It is unclear, if present, whether neutrophil senescence is a similarly negative process for the host however, *in vitro*, bench-aged neutrophils demonstrate changes in micro-RNA expression (epigenetic changes) which control chemokine and cytokine signaling, small GTPase activity and regulation of the actin cytoskeleton<sup>66</sup>, and these processes are implicated in cell migration, degranulation and inflammation.

### **Neutrophil heterogeneity.**

The classical view of neutrophils states these cells have an effective but undifferentiated response to inflammation and significant potential for host injury. However, alongside a recognition that neutrophil functions may change as they age, there is growing recognition that these cells may be more diverse in phenotype than first thought. Neutrophils are transcriptionally active<sup>67</sup>, can release a wide array of context specific products<sup>68</sup> and have an adaptable lifespan depending on activation status and environmental circumstance<sup>69</sup>. Furthermore, an increasing number of neutrophil phenotypes have been identified in different experimental models, which seem able to display different functional characteristics. During a significant inflammatory challenge such as sepsis, immature neutrophils are released from the bone marrow. These cells can phagocytose, have a longer

life span, are more resistance to apoptosis but migrate less efficiently than mature granulocytes<sup>70</sup>.

However, neutrophil phenotypes do not merely reflect cellular aging and more functionally distinct phenotypes have also been described<sup>71</sup>. Although our understanding of the nature of these phenotypes is incomplete, it appears that different phenotypes are present at different stages of the inflammatory process. In models of acute lung injury, there appear to be two waves of neutrophil recruitment; the first is the classical and pro-inflammatory neutrophil, as described above, which has been associated with significant tissue damage<sup>72</sup>. The second wave appears to be a pro-angiogenic neutrophil<sup>73</sup>, which is found in hypoxic tissues, can be identified by being CD49d<sup>+</sup>VEGFR1<sup>high</sup>CXCR4<sup>high</sup>, is characterized by increased MMP-9 release<sup>74,75</sup> and has been associated with improved clinical outcomes<sup>76</sup>.

There are also descriptions of anti-inflammatory neutrophils including low-density neutrophils (LDN) that appear in transient inflammation and during tumor clearance<sup>77</sup> and neutrophils which appear to modulate T cell function via Mac 1 signaling, leading to immune suppression<sup>78</sup>. Not all neutrophils stay in tissues for clearance by efferocytosis, and a subset have been shown to “reverse transmigrate” back into the systemic circulation<sup>79</sup>. How neutrophil populations change to respond to environmental challenges is unclear and it is yet unknown whether any of these phenotypes represent “senescent” neutrophils but population plasticity is well recognized in T cells<sup>80</sup> and monocytes/macrophages<sup>81</sup> and might also occur in neutrophils.

### **Neutrophils from the ageing host**

There are many theories of why we age as an organism but host and cellular aging appear inextricably linked. It has been hypothesised that host aging represents a failure of somatic maintenance, resulting in a build-up of cellular damage<sup>82</sup> and most models of aging include an increased presence of senescent cells which have been linked to organ dysfunction, disease and poor host outcomes<sup>83</sup>. Even in health, the aging host represents a pro-inflammatory environment for immune cells, with greater circulating levels of a number of cytokines including IL-6, Tumor necrosis factor (TNF) $\alpha$  and IL- $\beta$ <sup>84</sup> which might impact on cellular functions.

In health, neutrophil numbers do not change as we age<sup>85</sup>, however, during severe infections, older patients can exhibit a neutropenia, due to the blunted response of neutrophil progenitors to granulocyte colony stimulating factor (G-CSF) with age, which in health is compensated for by Granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-3<sup>86,87</sup>. Furthermore, the ability to delay apoptosis in response to survival signals (GM-CSF, Interferon-1) at the site of inflammation is impaired<sup>88</sup> and thus prolonged infectious insults can result in the double challenge of a reduction in mobilisation of immature neutrophil from the bone marrow without an increase in the survival of activated, mature cells.

Neutrophils donated from an old host often show a functional decline. Neutrophil adhesion to the endothelium appears unaltered in an aging population<sup>89,90</sup>, suggesting that extravasation of neutrophils is unchanged in old age but chemotaxis appears impaired with age<sup>91,92</sup>. We have published evidence that neutrophils from older donors are less able to accurately migrate due to dysregulated and excessive PI3K activity<sup>93</sup> and although there is a decline from middle age, the deficit is most apparent after people reach their sixth decade of life. This is associated with an increase in primary granule mobilization and increased neutrophil proteinase activity, as demonstrated by higher levels of CD63 on the surface of neutrophils and increased neutrophil elastase specific degradation products<sup>93</sup>, supporting increased degranulation. There is a reduction in phagocytic ability for opsonized bacteria<sup>90,94,95</sup>, especially to *staphylococcus aureus*<sup>85</sup>, an organism the elderly are notoriously susceptible to. CD16 levels are reduced with age, impacting Fc-mediated phagocytosis<sup>94</sup> and Fc Receptor triggered oxidative burst<sup>96</sup>. This phagocytic deficit is not mirrored in un-opsonized bacteria<sup>97</sup>, suggesting the receptors of innate recognition (e.g. CD14) are not affected by aging<sup>95</sup>. Finally, NET release are reduced in cells isolated from old subjects, in response to a number of physiological and pathological stimuli including lipopolysaccharide (LPS) and interleukin-8 (IL-8)<sup>98</sup>.

It is unclear why neutrophils from old donors have these functional deficits but it has been hypothesized that the low-grade inflammatory systemic environment seen with aging<sup>84,99</sup> may lead to epigenetic changes in cells, such as DNA methylation (which has been shown to

vary widely between individuals<sup>100</sup>) which in turn may impact on cellular phenotype and function<sup>101</sup>. These changes might alter the responses of immune cells to further inflammatory stimuli in a negative manner, heightening the potential for microbial invasion and host damage<sup>102</sup>. Currently, this mechanism remains putative, demonstrated in a limited number of studies. Further studies, ideally longitudinal and therefore long-term, will be needed to firmly link epigenetic changes to alterations in cellular functions to clinical outcomes, but these will be challenging to perform.

### **The impact of disease on neutrophil functions with age.**

Should sustained inflammation be causally implicated in dysregulated neutrophil responses, one might predict that chronic inflammatory diseases would be associated with an even greater burden of cellular dysfunction compared to healthy aging, as they are associated with more inflammation. There is some evidence to support this theory.

Cardiovascular disease is associated with neutrophilic inflammation. Neutrophil recruitment is associated with impaired microvascular perfusion, left ventricular dilation and adverse cardiac events in patients treated for myocardial infarction<sup>103</sup>. Furthermore, myeloperoxidase (a marker of neutrophil activity) is increased acutely following myocardial damage and predicts outcomes<sup>103,104</sup>. Similar to acute lung injury, there appear to be two waves of neutrophil recruitment to areas of infarction, with the first wave being pro-inflammatory, causing tissue damage<sup>105</sup> and the second being pro-angiogenic, assisting with new vessel formation and tissue reperfusion<sup>106</sup>, perhaps reflecting two different phenotypes of neutrophils. In old adults, changes in the expression and function of neutrophil small GTPases (which act downstream to PI3K, already implicated in neutrophil dysfunction<sup>93</sup>) appear to favor tissue damage rather than repair, and are associated with an increased future risk of cardiovascular disease<sup>107</sup>. Accelerated epigenetic aging has been associated with cardiovascular risk<sup>108,109</sup>, with hypermethylation of the gene body of Rho GTPase-activating protein 24 (ARHGAP24) reported in old patients with heart failure<sup>110</sup> and DNA methylation in peripheral blood leukocytes related to atherosclerosis in older adults<sup>111</sup>. Together, these studies support a link between accelerated aging, epigenetic influences, neutrophil functions and cardiovascular outcomes.

Similarly, neutrophils from patients with Chronic Obstructive Pulmonary Disease (COPD), which is classically characterized by neutrophilic inflammation<sup>10</sup> demonstrate some of the functional deficits present in the aging host. This includes poor migratory accuracy causally associated with PI3K signaling<sup>112</sup> a pro-inflammatory phenotype<sup>113</sup> with evidence of increased degranulation<sup>114</sup> but crucially, these changes to neutrophil function appear at younger age than seen in healthy smoking controls<sup>112</sup>. Smoking cigarettes, a pro-inflammatory pastime and the greatest risk factor for COPD, is associated with an accelerated epigenetic aging profile<sup>115</sup> which in turn is associated with the onset of chronic lung diseases including COPD and lung cancer<sup>116</sup>.

Most studies have only compared one chronic disease state to health and it is unclear whether multi-morbidity might alter neutrophil functions more than a single disease state. However, studies of other aspects of cellular immunity and inflammation provide some insights which suggest this might be the case. Data from the U.S. National Health and Nutrition Examination Survey data collected between 1999 and 2008 described relationships between C-reactive protein, white blood cell count, segmented neutrophils percent, eosinophils percent and glycohemoglobin levels and the number of chronic diseases present in an individual<sup>117</sup>.

### **The role of infections**

Severe infections are associated with an even greater burden of inflammation than chronic diseases, albeit (usually) over a shorter period of time and one might see changes in cellular phenotypes after these infective events.

A number of studies have highlighted the increased inflammatory burden experienced by older people during an infection. Systemic pro-inflammatory cytokines and chemokines including (for example) CXCL8, TNF $\alpha$ , IL-1 $\beta$  and IL-6 have been shown to be increased in a sustained manner during episodes of severe infections and sepsis in old adults compared to younger adults experiencing similar infective events<sup>118,119</sup>.

Despite this pro-inflammatory state during infections, cross sectional data suggest that the immune response is impaired, leading to a paradoxical state of enhanced systemic

inflammation but also reduced immune capacity. There is a stepwise decrease in the effectiveness of the immune response and in particular the accuracy of neutrophil migration, as the severity of the infectious insult progresses in old patients (from a simple lung infection to pneumonia with sepsis), but this is not seen in young adults until the development of sepsis<sup>120</sup>. Of interest, the highly blunted neutrophil responses described in sepsis<sup>121</sup> and implicated in multi-organ failure<sup>122</sup> occur in both young and old adults, and are associated with sustained inflammation<sup>121</sup> with a reduction in anti-inflammatory signaling<sup>123</sup>. Neutrophil functions remain blunted at six weeks following the infective event, suggesting a prolonged period of immune suppression, but of note, this is only seen in old adults, as neutrophils from young donors recover to baseline functions within this time<sup>120</sup>. In support of this experimental finding, primary infections are often followed by secondary infections in old adults, associated with immune suppression and poorer clinical outcomes<sup>24</sup>.

Most studies of cell functions during infection have not considered multi-morbidity, but it is clear that patients with multiple co-morbidities have the worse clinical outcomes. Therefore, it is tempting to hypothesize that neutrophil functions may be further impaired.

In summary, current data support a similar alteration of neutrophil functions in a number of chronic inflammatory diseases and during infections in old age. Inflammation and epigenetic modifications are noted in some studies, and therefore might represent a shared mechanism of effect. However, current data is not prospective, and therefore it is unclear which came first; the aging, the altered neutrophil function, the illness (be that the chronic disease or severe infective event), the inflammation or the epigenetic change. It is also uncertain why neutrophil responses change with age and illness.

Neutrophils do not act in isolation, but represent a component of a (usually) highly coordinated immune response, and just as there is evidence of dysregulated neutrophil responses with age and disease, similar defects have been described with other cells of the immune system. For example, monocyte and macrophage function has been shown to be altered with age<sup>124</sup>, and in chronic disease such as COPD<sup>125</sup> where a reduction in anti-aging molecules has been described<sup>126</sup>.

In some age-related chronic diseases, a reduction in the plasticity of immune cell populations has been described, which might favour sustained inflammation. For example, in Rheumatoid arthritis, this has been described within joint T cell populations<sup>127</sup> and in COPD, monocyte derived macrophages have been shown to retain a pro-inflammatory profile; losing their ability to change to an anti-inflammatory phenotype, in contrast with cells isolated from non-COPD controls<sup>128</sup>. With aging, there might be epigenetic-driven changes in either the proportion of different phenotypic populations of neutrophils, or an inability to change phenotype during specific challenges. Chronic disease and severe infections might amplify these responses, creating a negative cycle of further cellular dysfunction (see figure 2). If this were the case, it is likely that all facets of the immune system would be affected and indeed, there is significant evidence to support the notion that this is not a “one cell” problem.

### **Targeted treatments**

Further work is needed to understand the mechanisms underpinning the decline in the immune system with age, but already some potential targets have been identified which may offer therapeutic relief by targeting immune responses.

PI3K has been implicated in the neutrophil migratory defect seen with ageing, and PI3K inhibitors have been shown to restore neutrophil migratory accuracy *in vitro*, especially Class 1 delta and gamma isoforms<sup>93</sup> which are enriched in neutrophils and implicated in cell migration. Early phase clinical trials of a PI3K delta inhibitor to improve inflammation are already underway in COPD and bronchiectasis, a suppurative lung disease<sup>129</sup>.

Nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) has been shown to be decreased in a number of aging co-morbidities including COPD<sup>130</sup>. Low levels of Nrf2 would contribute to oxidative stress, and pharmacological activation of Nrf2 by sulforaphane, a potent activator of Nrf2, has been shown to improve facets of the immune response in innate immune cells, including enhancing the phagocytosis of bacteria<sup>131</sup>.

If mechanisms are linked by the common theme of aging in multi-morbidity, therapeutic targets could cross disease silos. Recently, we have shown that 80mg Simvastatin (a HMG Co-A reductase inhibitor classically used to reduce cholesterol levels) once daily for two weeks restored neutrophil migratory accuracy in a randomised, double-blinded clinical trial in healthy old adults. The same dose had the same restorative effect on neutrophil migration during pneumonia but not sepsis and not in young adults *in vitro*<sup>120</sup>. This builds on a body of evidence suggesting that statins may positively impact on patient outcomes during infections, but only when used in old adults, at high dose, and before the onset of severe sepsis<sup>132</sup>. The exact mechanism of effect is unclear, but statins are known to alter small GTPase activity, which in turn would impact on cellular polarity and migrational accuracy<sup>133</sup>. We have also reported similar positive effects on immune function when statins were used in COPD<sup>134</sup> and there are on-going studies of statins being used in inflammatory conditions far removed from cholesterol modification, including uveitis<sup>135</sup>, where neutrophils are implicated in tissue damage<sup>136</sup>.

There is great interest in the development of drugs to maintain healthy aging, and this is an area of pharmacodiscovery which needs to expand.

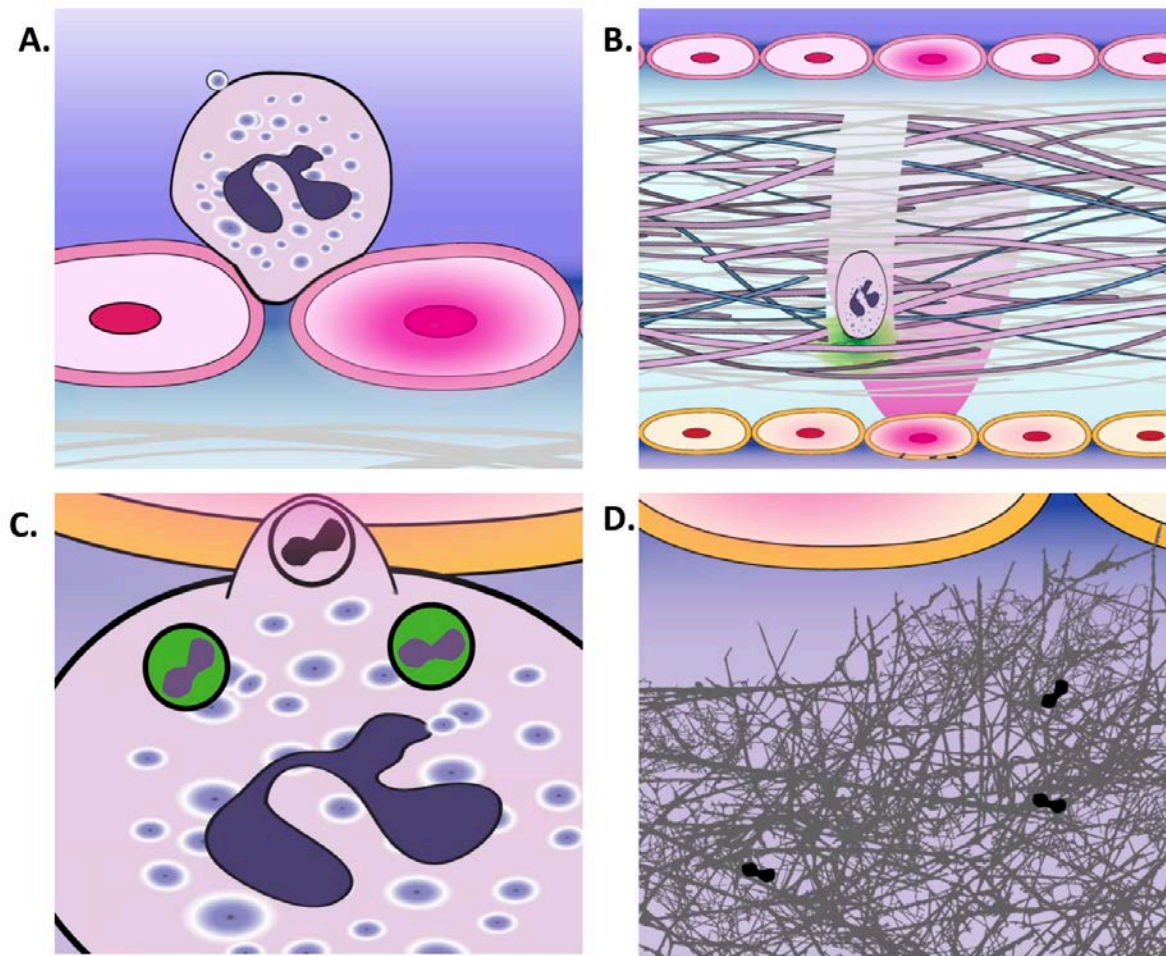
## **Conclusion.**

Multi-morbidity and the sequelae of surviving severe infections are associated with a significant burden of ill health in old adults. Many of these conditions share a sustained and damaging inflammatory response with innate immunity, and in particular neutrophils, implicated in disease processes. There is increasing evidence to support a global decline in immune cell function in old age, which appears even more compromised in the presence of inflammatory challenges such as chronic disease and acute infections. Recent studies of neutrophils in (mainly murine) disease models support there being different phenotypes of neutrophils, which present to tissues depending on the inflammatory challenge. In some studies, these cells have been termed “senescent”. Being a non-dividing cell, it is unclear whether neutrophils can enter a senescent state, as described in other cells, and whether they can remain pro-inflammatory without entering apoptosis or being cleared by macrophages. More studies are needed to understand neutrophil responses and phenotypes in age, disease and multi-morbidity, but emerging evidence suggest that we can



target these processes, and this may give us new therapeutic options to improve older patient outcomes.

## Figures



**Figure 1. Neutrophil functions in health and old age**

### **Legend.**

- A.** Systemic neutrophils adhere and transmigrate past endothelial cells to enter the extracellular matrix. This process is thought to be unaltered with age.
- B.** Once extravasation has taken place, neutrophils follow chemoattractant gradients formed by chemokines to move towards the inflammatory source. They release proteinases and ROS (shown in green) to degrade a pathway through the dense extracellular matrix. In old age, this pathway is less direct, with reduced accuracy of migration.
- C.** Once at the site of infection, neutrophils phagocytose bacteria into the phagosome, which then matures into a bactericidal lysophagosome (shown in green) by combining the vesicle containing the bacteria with the toxic contents of neutrophil granules. In age, phagocytosis of opsonized bacteria is reduced, with reduced expression of CD16.
- D.** Once the phagocytic capacity of neutrophils has been reached, they extrude their DNA contents which become coated in bacteriocidal proteins, including neutrophil elastase and myeloperoxidase. NETs can be greater than 10x larger than the cell itself. In old age, NET release is reduced, thought to be moderated by a reduction in ROS generation, a critical step in NET formation.

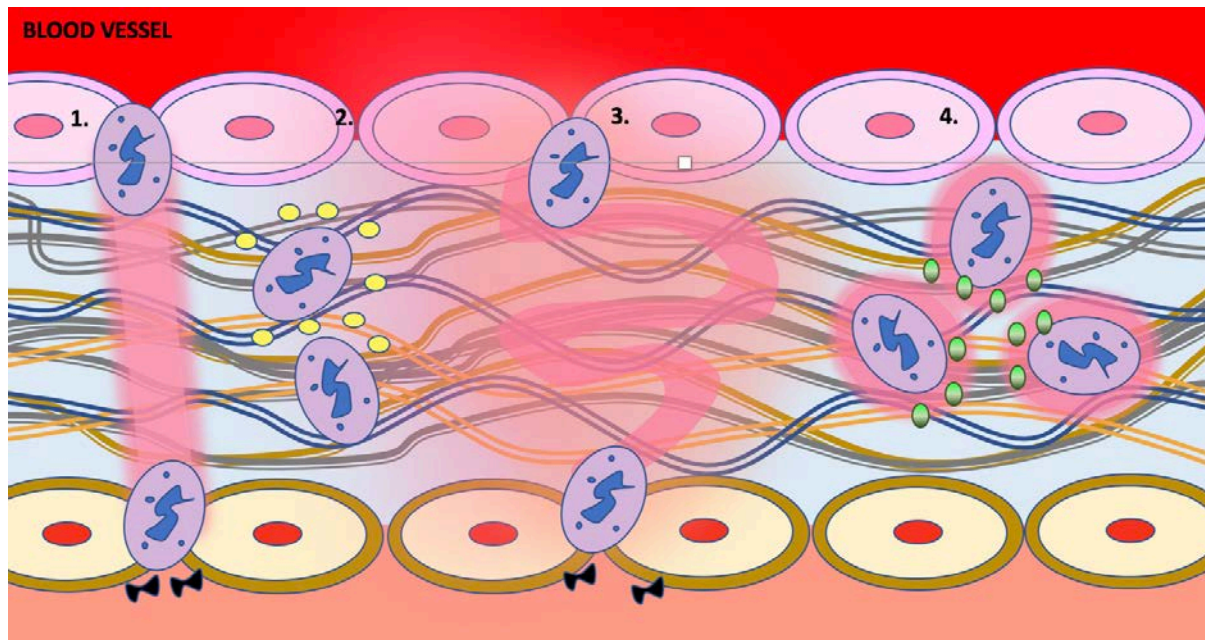


Figure 2. Reduced neutrophil plasticity with age: a potential mechanism to link age, inflammation and chronic disease?

Legend.

1. In health, during an infectious challenge, neutrophils migrate accurately to the infected or inflamed tissue. Once there, they clear bacteria by phagocytosis and in turn are cleared by macrophages in a process termed efferocytosis.
2. There is increasing recognition that other neutrophil phenotypes may also contribute to tissue repair. Some neutrophils appear to migrate away from inflammation, and return to the margined pool (termed reverse transmigration), others are anti-inflammatory or pro-angiogenic, releasing MMP-9 to promote new vessel formation and clear inflammation (as shown here).
3. With increasing age, the presence of low grade systemic inflammation may reduce the diversity of neutrophilic responses, so that only a pro-inflammatory and injurious phenotype is supported. Neutrophil migratory accuracy is reduced, as is the ability to clear opsonised bacteria.
4. These pro-inflammatory cells lead to further tissue inflammation and increase the potential for subsequent infections and organ dysfunction by causing tissue damage.

**References**

1. Parker MG, Thorslund M. Health trends in the elderly population: getting better and getting worse. *Gerontologist*. 2007;47(0016-9013 (Print)):150 - 158.
2. Organization.; WH. Noncommunicable diseases. *World Health Organisation*. 2017;<http://www.who.int/mediacentre/factsheets/fs355/en/>(Updated June 2017):DOA 14th August 2017.
3. Mannino DM, Thorn D, Swensen A, Holguin F. Prevalence and outcomes of diabetes, hypertension and cardiovascular disease in COPD. *The European respiratory journal*. 2008;32(4):962-969.

4. Lloyd-Jones D, Adams R, Carnethon M, et al. Heart Disease and Stroke Statistics—2009 Update. *Circulation*. 2009;119(3):e21.
5. Hanania NA, Sharma G, Sharafkhaneh A. COPD in the elderly patient. *Semin Respir Crit Care Med*. 2010;31(5):596-606.
6. Barnett K, Mercer SW, Norbury M, Watt G, Wyke S, Guthrie B. Epidemiology of multimorbidity and implications for health care, research, and medical education: a cross-sectional study. *The Lancet*. 380(9836):37-43.
7. Rizzuto D, Melis RJF, Angleman S, Qiu C, Marengoni A. Effect of Chronic Diseases and Multimorbidity on Survival and Functioning in Elderly Adults. *J Am Geriatr Soc*. 2017;65(5):1056-1060.
8. World Health Organization. Multimorbidity: technical series on safer primary care. Geneva. 2016;Licence: CC BY-NC-SA 3.0  
IGO.(<http://apps.who.int/iris/bitstream/10665/252275/1/9789241511650-eng.pdf>):DOA 9th November 2017.
9. Excellence.; NifHaC. Multi-morbidity: Clinical assessment and management. *BNICE guideline NG56*. 2016;[https://www.nice.org.uk/guidance/ng56\(DOA](https://www.nice.org.uk/guidance/ng56(DOA) 14th August 2017).
10. Stone H, McNab G, Wood AM, Stockley RA, Sapey E. Variability of sputum inflammatory mediators in COPD and alpha1-antitrypsin deficiency. *The European respiratory journal*. 2012;40(3):561-569.
11. Ayilavarapu S, Kantarci A, Fredman G, et al. Diabetes-Induced Oxidative Stress Is Mediated by Ca(2+)-Independent Phospholipase A2 in Neutrophils. *Journal of immunology (Baltimore, Md : 1950)*. 2010;184(3):1507-1515.
12. Ginaldi L, Di Benedetto MC, De Martinis M. Osteoporosis, inflammation and ageing. *Immunity & ageing : I & A*. 2005;2:14-14.
13. Bruunsgaard H, Andersen-Ranberg K, Jeune B, Pedersen AN, Skinhoj P, Pedersen BK. A high plasma concentration of TNF-alpha is associated with dementia in centenarians. *J Gerontol A Biol Sci Med Sci*. 1999;54(7):M357-364.
14. Jackson CA, Jones M, Tooth L, Mishra GD, Byles J, Dobson A. Multimorbidity patterns are differentially associated with functional ability and decline in a longitudinal cohort of older women. *Age and Ageing*. 2015;44(5):810-816.
15. Déruaz-Luyet A, Goran AA, Senn N, et al. Multimorbidity and patterns of chronic conditions in a primary care population in Switzerland: a cross-sectional study. *BMJ Open*. 2017;7(6).
16. Sevenoaks MJ, Stockley RA. Chronic obstructive pulmonary disease, inflammation and co-morbidity - a common inflammatory phenotype? *Respir Res*. 2006;7:70 -76.
17. Lim WS, van der Eerden MM, Laing R, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. *Thorax*. 2003;58:377 - 382.
18. Broulette J, Yu H, Pyenson B, Iwasaki K, Sato R. The Incidence Rate and Economic Burden of Community-Acquired Pneumonia in a Working-Age Population. *American Health & Drug Benefits*. 2013;6(8):494-503.
19. Fein AM. Pneumonia in the elderly: Overview of diagnostic and therapeutic approaches. *Clin Infect Dis*. 1999;28:726 - 729.
20. Lindenauer PK, Lagu T, Shieh M, Pekow PS, Rothberg MB. Association of diagnostic coding with trends in hospitalizations and mortality of patients with pneumonia, 2003-2009. *JAMA*. 2012;307(13):1405-1413.

21. Klevens RM, Edwards JR, Gaynes RP. The Impact of Antimicrobial-Resistant, Health Care–Associated Infections on Mortality in the United States. *Clinical Infectious Diseases*. 2008;47(7):927-930.
22. Restrepo MI, Faverio P, Anzueto A. Long-term prognosis in community-acquired pneumonia. *Current opinion in infectious diseases*. 2013;26(2):151-158.
23. Dick A, Liu H, Zwanziger J, et al. Long-term survival and healthcare utilization outcomes attributable to sepsis and pneumonia. *BMC Health Serv Res*. 2012;12:432-432.
24. van Vught LA, Klein Klouwenberg PM, Spitoni C, et al. Incidence, Risk Factors, and Attributable Mortality of Secondary Infections in the Intensive Care Unit After Admission for Sepsis. *JAMA*. 2016;315(1538-3598 (Electronic)):1469-1479.
25. Stockley JA, Walton GM, Lord JM, Sapey E. Aberrant neutrophil functions in stable chronic obstructive pulmonary disease: the neutrophil as an immunotherapeutic target. *International immunopharmacology*. 2013;17(4):1211-1217.
26. de Boer OJ, Li X, Teeling P, et al. Neutrophils, neutrophil extracellular traps and interleukin-17 associate with the organisation of thrombi in acute myocardial infarction. *Thromb Haemost*. 2013;109(2):290-297.
27. Alba-Loureiro TC, Munhoz C.D., Martins J.O., et al. Neutrophil function and metabolism in individuals with diabetes mellitus. *Braz J Med Biol Res*. 2007;40(0100-879X (Print)):1037-1044.
28. Wilson D, Jackson T, Sapey E, Lord JM. Frailty and sarcopenia: The potential role of an aged immune system. *Ageing Research Reviews*. 2017;36(Supplement C):1-10.
29. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. *Annual review of immunology*. 2012;30:459-489.
30. Galli SJ, Borregaard N, Wynn TA. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. *Nature immunology*. 2011;12(11):1035-1044.
31. Borregaard N. Neutrophils, from marrow to microbes. *Immunity*. 2010;33(5):657-670.
32. Faurschou M, Borregaard N. Neutrophil granules and secretory vesicles in inflammation. *Microbes and infection*. 2003;5(14):1317-1327.
33. Klebanoff SJ. Myeloperoxidase. *Proceedings of the Association of American Physicians*. 1999;111(5):383-389.
34. Cramer E, Pryzwansky KB, Villeval JL, Testa U, Breton-Gorius J. Ultrastructural localization of lactoferrin and myeloperoxidase in human neutrophils by immunogold. *Blood*. 1985;65(2):423-432.
35. Oram JD, Reiter B. Inhibition of bacteria by lactoferrin and other iron-chelating agents. *Biochim Biophys Acta*. 1968;170(2):351-365.
36. Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood*. 1997;89(10):3503-3521.
37. Segal AW. How neutrophils kill microbes. *Annual review of immunology*. 2005;23:197-223.
38. Liou TG, Campbell EJ. Quantum proteolysis resulting from release of single granules by human neutrophils: a novel, nonoxidative mechanism of extracellular proteolytic activity. *J Immunol*. 1996;157(6):2624-2631.
39. Summers C, Rankin SM, Condliffe AM, Singh N, Peters AM, Chilvers ER. Neutrophil kinetics in health and disease. *Trends Immunol*. 2010;31(8):318-324.

40. Summers C, Singh NR, White JF, et al. Pulmonary retention of primed neutrophils: a novel protective host response, which is impaired in the acute respiratory distress syndrome. *Thorax*. 2014;doi: 10.1136/thoraxjnl-2013-204742. [Epub ahead of print].
41. Furze RC, Rankin SM. The role of the bone marrow in neutrophil clearance under homeostatic conditions in the mouse. *FASEB J*. 2008;22(1530-6860 (Electronic)):3111 - 3119.
42. Raman D, Sai J, Neel NF, Chew CS, Richmond A. LIM and SH3 Protein-1 Modulates CXCR2 mediated Cell Migration. *PLoS ONE*. 2010;5:e100050. doi:100010.101371/journal.pone.0010050.
43. Hannigan MO, Huang CK, Wu DQ. Roles of PI3K in neutrophil function. *Curr Top Microbiol Immunol*. 2003;282:165 - 175.
44. Botelho RJ, Teruel M, Dierckman R, et al. Localised biphasic changes in phosphatidylinositol-4,5-biphosphate at sites of phagocytosis. *JCB*. 2000;151:1353-1368.
45. Ito N, Yokomizo T, Sasaki T, et al. Requirement of phosphatidylinositol 3 kinase activation and calcium influx for leukotrine B4-induced enzyme release. *J Biol Chem*. 2002;277:44898-44904.
46. Yoo SK, Deng Q, Cavnar PJ, Wu YI, Hahn KM, Huttenlocher A. Differential regulation of protrusion and polarity by PI3K during neutrophil motility in live zebrafish *Dev Cell*. 2010;18:226 - 236.
47. Burridge K, Doughman R. Front and back by Rho and Rac. *Nat Cell Biol*. 2006;8:781 - 782.
48. Charest PG, Firtel RA. Big role for small GTPases in the control of directed cell movement. *Biochem J*. 2007;401:377 - 390.
49. Cepinskas G, Sandig M, Kvietys PR. PAF induced elastase dependent neutrophil transendothelial migration is associated with the mobilisation of elastase to the neutrophil surface and localised to the migrating front. *J Cell Sci*. 1999;112:1937 - 1945.
50. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: From mechanisms to disease. *Ann Rev Immunol*. 2012;30:459 - 489.
51. Hayashi F, Means TK, Luster AD. Toll-like receptors stimulate human neutrophil function. *Blood*. 2003;102(7):2660-2669.
52. Lee WL, Harrison RE, Grinstein S. Phagocytosis by neutrophils. *Microbes Infect*. 2003;5(14):1299-1306.
53. Kovari DT, Wei W, Toro J-S, Fogg RE, Porter K, Curtis JE. Frustrated Phagocytic Spreading Dynamics End in Distinct Non-Muscle Myosin II Dependent Contraction. *Biophysical Journal*. 2016;110(3):621a.
54. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004;303(5663):1532-1535.
55. Liu S, Su X, Pan P, et al. Neutrophil extracellular traps are indirectly triggered by lipopolysaccharide and contribute to acute lung injury. *Scientific Reports*. 2016;6:37252.
56. Owen CA, Campbell MA, Sannes PL, Boukedes SS, Campbell EJ. Cell surface bound elastase and cathepsin G on human neutrophils; a novel, non-oxidative mechanism by which neutrophils focus and preserve catalytic activity of serine proteinases. *J Cell Biol*. 1995;131:775 - 789.

57. Sapey E, Stockley RA. Red, amber and green: the role of the lung in de-priming active systemic neutrophils. *Thorax*. 2014;69(7):606-608.
58. Eash KJ, Greenbaum AM, Gopalan PK, Link DC. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *The Journal of Clinical Investigation*. 2010;120(7):2423-2431.
59. Casanova-Acebes M, Pitaval C, Weiss LA, et al. Rhythmic modulation of the hematopoietic niche through neutrophil clearance. *Cell*. 2013;153(5):1025-1035.
60. Zhang D, Chen G, Manwani D, et al. Neutrophil ageing is regulated by the microbiome. *Nature*. 2015;525(7570):528-532.
61. Salati S, Bianchi E, Zini R, et al. Eosinophils, but not neutrophils, exhibit an efficient DNA repair machinery and high nucleolar activity. *Haematologica*. 2007;92(10):1311.
62. Adrover JM, Nicolas-Avila JA, Hidalgo A. Aging: A Temporal Dimension for Neutrophils. *Trends Immunol*. 2016;37(5):334-345.
63. Martin C, Burdon PCE, Bridger G, Gutierrez-Ramos J-C, Williams TJ, Rankin SM. Chemokines Acting via CXCR2 and CXCR4 Control the Release of Neutrophils from the Bone Marrow and Their Return following Senescence. *Immunity*. 2003;19(4):583-593.
64. Uhl B, Vadlau Y, Zuchtriegel G, et al. Aged neutrophils contribute to the first line of defense in the acute inflammatory response. *Blood*. 2016;128(19):2327-2337.
65. Muñoz-Espín D, Serrano M. Cellular senescence: from physiology to pathology. *Nature Reviews Molecular Cell Biology*. 2014;15:482.
66. Ward JR, Heath PR, Catto JW, Whyte MKB, Milo M, Renshaw SA. Regulation of Neutrophil Senescence by MicroRNAs. *PLoS ONE*. 2011;6(1):e15810.
67. Yost CC, Denis MM, Lindemann S, et al. Activated Polymorphonuclear Leukocytes Rapidly Synthesize Retinoic Acid Receptor- $\alpha$ . *The Journal of Experimental Medicine*. 2004;200(5):671.
68. Tecchio C, Micheletti A, Cassatella MA. Neutrophil-Derived Cytokines: Facts Beyond Expression. *Front Immunol*. 2014;5:508.
69. Walmsley SR, Print C, Farahi N, et al. Hypoxia-induced neutrophil survival is mediated by HIF-1 $\alpha$ -dependent NF- $\kappa$ B activity. *The Journal of Experimental Medicine*. 2005;201(1):105-115.
70. Drifte G, Dunn-Siegrist I, Tissières P, Pugin J. Innate Immune Functions of Immature Neutrophils in Patients With Sepsis and Severe Systemic Inflammatory Response Syndrome\*. *Crit Care Med*. 2013;41(3):820-832.
71. Silvestre Roig C, Hidalgo A, Soehnlein O. Neutrophil heterogeneity: implications for homeostasis and pathogenesis. *Blood*. 2016;127:2173-2181.
72. Yang K-Y, Arcaroli JJ, Abraham E. Early Alterations in Neutrophil Activation Are Associated with Outcome in Acute Lung Injury. *American Journal of Respiratory and Critical Care Medicine*. 2003;167(11):1567-1574.
73. Bekes EM, Schweighofer B, Kupriyanova TA, et al. Tumor-recruited neutrophils and neutrophil TIMP-free MMP-9 regulate coordinately the levels of tumor angiogenesis and efficiency of malignant cell intravasation. *Am J Pathol*. 2011;179(1525-2191 (Electronic)):1455 - 1470.
74. Christoffersson G, Vågesjö E, Vandooren J, et al. VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue. *Blood*. 2012;120(23):4653.



75. Massena S, Christoffersson G, Vågesjö E, et al. Identification and characterization of VEGF-A-responsive neutrophils expressing CD49d, VEGFR1, and CXCR4 in mice and humans. *Blood*. 2015;126(17):2016.
76. Blázquez-Prieto J, López-Alonso I, Amado-Rodríguez L, et al. Impaired lung repair during neutropenia can be reverted by matrix metalloproteinase-9. *Thorax*. 2017;pii: thoraxjnl-2017-210105.:doi: 10.1136/thoraxjnl-2017-210105. [Epub ahead of print].
77. Sagiv Jitka Y, Michaeli J, Assi S, et al. Phenotypic Diversity and Plasticity in Circulating Neutrophil Subpopulations in Cancer. *Cell Reports*. 2015;10(4):562-573.
78. Pillay J, Kamp VM, van Hoffen E, et al. A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. *J Clin Invest*. 2012;122(1):327-336.
79. Buckley CD, Ross EA, McGettrick HM, et al. Identification of a phenotypically and functionally distinct population of long-lived neutrophils in a model of reverse endothelial migration. *Journal of Leukocyte Biology*. 2006;79(2):303-311.
80. Magombedze G, Reddy P, Eda S, Ganusov V. Cellular and population plasticity of helper CD4+ T cell responses. *Frontiers in Physiology*. 2013;4:206.
81. Das A, Sinha M, Datta S, et al. Monocyte and Macrophage Plasticity in Tissue Repair and Regeneration. *Am J Pathol*. 2015;185(10):2596-2606.
82. Kirkwood TBL. Understanding the Odd Science of Aging. *Cell*. 2005;120(4):437-447.
83. Childs BG, Durik M, Baker DJ, van Deursen JM. Cellular senescence in aging and age-related disease: from mechanisms to therapy. *Nature Medicine*. 2015;21:1424.
84. Franceschi C, Campisi J. Chronic Inflammation (Inflammaging) and Its Potential Contribution to Age-Associated Diseases. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*. 2014;69(Suppl 1):S4-S9.
85. Wenisch C, Patruta S, Daxböck F, Krause R, Horl W. Effect of age on human neutrophil function. *J Leukoc Biol*. 2000;67(1):40-45.
86. Chatta GS, Andrews RG, Rodger E, Schrag M, Hammond WP, Dale DC. Hematopoietic progenitors and aging: alterations in granulocytic precursors and responsiveness to recombinant human G-CSF, GM-CSF, and IL-3. *J Gerontol*. 1993;48(5):M207-212.
87. Lord JM, Butcher S, Killampali V, Lascelles D, Salmon M. Neutrophil ageing and immunesenescence. *Mechanisms of ageing and development*. 2001;122(14):1521-1535.
88. Fortin CF, Larbi A, Dupuis G, Lesur O, Fulop T, Jr. GM-CSF activates the Jak/STAT pathway to rescue polymorphonuclear neutrophils from spontaneous apoptosis in young but not elderly individuals. *Biogerontology*. 2007;8(2):173-187.
89. MacGregor RR, Shalit M. Neutrophil function in healthy elderly subjects. *J Gerontol*. 1990;45(2):M55-60.
90. Butcher S, Chahel H, Lord JM. Ageing and the neutrophil: no appetite for killing? *Immunology*. 2000;100(4):411-416.
91. Fulop T, Larbi A, Douziech N, et al. Signal transduction and functional changes in neutrophils with aging. *Aging Cell*. 2004;3(4):217-226.
92. Niwa Y, Kasama T, Miyachi Y, Kanoh T. Neutrophil chemotaxis, phagocytosis and parameters of reactive oxygen species in human aging: cross-sectional and longitudinal studies. *Life Sci*. 1989;44(22):1655-1664.
93. Sapey E, Greenwood H, Walton G, et al. Phosphoinositide 3-kinase inhibition restores neutrophil accuracy in the elderly: toward targeted treatments for immunosenescence. *Blood*. 2014;123(2):239-248.



94. Butcher SK, Chahal H, Nayak L, et al. Senescence in innate immune responses: reduced neutrophil phagocytic capacity and CD16 expression in elderly humans. *J Leukoc Biol.* 2001;70(6):881-886.
95. Weiskopf D, Weinberger B, Grubeck-Loebenstien B. The aging of the immune system. *Transpl Int.* 2009;22(11):1041-1050.
96. Fülöp T, Fóris G, Wórum I, Leövey A. Age-dependent alterations of Fc gamma receptor-mediated effector functions of human polymorphonuclear leucocytes. *Clin Exp Immunol.* 1985;61(2):425-432.
97. Emanuelli G, Lanzio M, Anfossi T, Romano S, Anfossi G, Calcamuggi G. Influence of age on polymorphonuclear leukocytes in vitro: phagocytic activity in healthy human subjects. *Gerontology.* 1986;32(6):308-316.
98. Hazeldine J, Harris P, Chapple IL, et al. Impaired neutrophil extracellular trap formation: a novel defect in the innate immune system of aged individuals. *Aging cell.* 2014;13(4):690-698.
99. De Martinis M, Franceschi C, Monti D, Ginaldi L. Inflammaging and life long antigenic load as major determinants of aging rate and longevity. . *FEBS Lett.* 2005;579:2035 - 2039.
100. Chatterjee A, Stockwell PA, Rodger EJ, et al. Genome-wide DNA methylation map of human neutrophils reveals widespread inter-individual epigenetic variation. *Scientific Reports.* 2015;5:17328.
101. Chen L, Ge B, Casale FP, et al. Genetic Drivers of Epigenetic and Transcriptional Variation in Human Immune Cells. *Cell.* 2016;167(5):1398-1414.e1324.
102. Ecker S, Chen L, Pancaldi V, et al. Genome-wide analysis of differential transcriptional and epigenetic variability across human immune cell types. *Genome Biology.* 2017;18(1):18.
103. Takahashi T, Hiasa Y, Ohara Y, et al. Relationship of admission neutrophil count to microvascular injury, left ventricular dilation, and long-term outcome in patients treated with primary angioplasty for acute myocardial infarction. *Circ J.* 2008;72(6):867-872.
104. Kaya MG, Yalcin R, Okyay K, et al. Potential Role of Plasma Myeloperoxidase Level in Predicting Long-Term Outcome of Acute Myocardial Infarction. *Texas Heart Institute Journal.* 2012;39(4):500-506.
105. Litt MR, Jeremy RW, Weisman HF, Winkelstein JA, Becker LC. Neutrophil depletion limited to reperfusion reduces myocardial infarct size after 90 minutes of ischemia. Evidence for neutrophil-mediated reperfusion injury. *Circulation.* 1989;80(6):1816.
106. Iyer RP, Jung M, Lindsey ML. MMP-9 signaling in the left ventricle following myocardial infarction. *American Journal of Physiology - Heart and Circulatory Physiology.* 2016;311(1):H190.
107. Florian MC, Klenk J, Marka G, et al. Expression and activity of the small RhoGTPase Cdc42 in blood cells of older adults are associated with age and cardiovascular disease. LID - 10.1093/gerona/glx091 [doi]. *J Gerontol A Biol Sci Med Sci.* 2017;doi: 10.1093/gerona/glx091. [Epub ahead of print](1758-535X (Electronic)).
108. Horvath S, Gurven M, Levine ME, et al. An epigenetic clock analysis of race/ethnicity, sex, and coronary heart disease. *Genome Biology.* 2016;17:171.
109. Perna L, Zhang Y, Mons U, Holleczeck B, Saum K-U, Brenner H. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. *Clinical Epigenetics.* 2016;8(1):64.

110. Movassagh M, Choy M-K, Goddard M, Bennett MR, Down TA, Foo RSY. Differential DNA Methylation Correlates with Differential Expression of Angiogenic Factors in Human Heart Failure. *PLoS ONE*. 2010;5(1):e8564.
111. Castro R, Rivera I, Struys EA, et al. Increased Homocysteine and S-Adenosylhomocysteine Concentrations and DNA Hypomethylation in Vascular Disease. *Clinical Chemistry*. 2003;49(8):1292.
112. Sapey E, Stockley JA, Greenwood H, et al. Behavioral and structural differences in migrating peripheral neutrophils from patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*. 2011;183(9):1176-1186.
113. Loi ALT, Hoonhorst S, van Aalst C, et al. Proteomic profiling of peripheral blood neutrophils identifies two inflammatory phenotypes in stable COPD patients. *Respiratory Research*. 2017;18(1):100.
114. Carter RI, Ungurs MJ, Mumford RA, Stockley RA. A $\alpha$ -Val360: a marker of neutrophil elastase and COPD disease activity. *European Respiratory Journal*. 2012;41(1):31.
115. DeMeo DL, Morrow JD, Hersh CP, et al. Accelerated Ticking of the Epigenetic Clock in Smokers with and Without COPD. *D91. GENETICS AND GENOMICS OF COPD*: American Thoracic Society; 2016:A7479-A7479.
116. Levine ME, Hosgood HD, Chen B, Absher D, Assimes T, Horvath S. DNA methylation age of blood predicts future onset of lung cancer in the women's health initiative. *Aging (Albany NY)*. 2015;7(9):690-700.
117. Stepanova M, Rodriguez E, Birerdinc A, Baranova A. Age-independent rise of inflammatory scores may contribute to accelerated aging in multi-morbidity. *Oncotarget*. 2015;6(3):1414-1421.
118. Ginde AA, Blatchford PJ, Trzeciak S, et al. Age-related differences in biomarkers of acute inflammation during hospitalization for sepsis. *Shock (Augusta, Ga)*. 2014;42(2):99-107.
119. Inoue S, Suzuki K, Komori Y, et al. Persistent inflammation and T cell exhaustion in severe sepsis in the elderly. *Crit Care*. 2014;18(3):R130-R130.
120. Sapey E, Patel JM, Greenwood HL, et al. Pulmonary Infections in the Elderly Lead to Impaired Neutrophil Targeting, Improved by Simvastatin. LID - 10.1164/rccm.201704-0814OC [doi]. *Am J Respir Crit Care Med*. 2017;doi: 10.1164/rccm.201704-0814OC. [Epub ahead of print](1535-4970 (Electronic)).
121. Arraes SMA, Freitas MS, da Silva SV, et al. Impaired neutrophil chemotaxis in sepsis associates with GRK expression and inhibition of actin assembly and tyrosine phosphorylation. *Blood*. 2006;108(9):2906-2913.
122. Brown KA, Brain SD, Pearson JD, Edgeworth JD, Lewis SM, Treacher DF. Neutrophils in development of multiple organ failure in sepsis. *The Lancet*. 2006;368(9530):157-169.
123. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: Results of the genetic and inflammatory markers of sepsis (genims) study. *Archives of Internal Medicine*. 2007;167(15):1655-1663.
124. Prattichizzo F, Bonafè M, Olivieri F, Franceschi C. Senescence associated macrophages and "macroph-aging": are they pieces of the same puzzle? *Aging (Albany NY)*. 2016;8(12):3159-3160.
125. Taylor AE, Finney-Hayward TK, Quint JK, et al. Defective macrophage phagocytosis of bacteria in COPD. *European Respiratory Journal*. 2010;35(5):1039.

126. Baker J, Colley T, Ito K, Barnes P. The key role of microRNA-34a in the reduction of sirtuin-1 in COPD. *European Respiratory Journal*. 2016;48(suppl 60).
127. Wang T, Sun X, Zhao J, et al. Regulatory T cells in rheumatoid arthritis showed increased plasticity toward Th17 but retained suppressive function in peripheral blood. *Annals of the Rheumatic Diseases*. 2015;74(6):1293.
128. Chana KK, Day AM, Ward AJN, Barnes PJ, Donnelly LE. Lack of macrophage plasticity in COPD. *European Respiratory Journal*. 2012;40(Suppl 56).
129. Doukas J, Eide L, Stebbins K, et al. Aerosolized Phosphoinositide 3-Kinase  $\gamma/\delta$  Inhibitor TG100-115 [3-[2,4-Diamino-6-(3-hydroxyphenyl)pteridin-7-yl]phenol] as a Therapeutic Candidate for Asthma and Chronic Obstructive Pulmonary Disease. *Journal of Pharmacology and Experimental Therapeutics*. 2009;328(3):758.
130. Ito K, Colley T, Mercado N. Geroprotectors as a novel therapeutic strategy for COPD, an accelerating aging disease. *International Journal of Chronic Obstructive Pulmonary Disease*. 2012;7:641-652.
131. Harvey CJ, Thimmulappa RK, Sethi S, et al. Nrf2-dependent Immunomodulation By Sulforaphane Improves Bacterial Phagocytosis In COPD Macrophages And Inhibits Bacterial Burden And Inflammation In Cigarette Smoke-exposed Mice. *B103. CHRONIC OBSTRUCTIVE PULMONARY DISEASE: INSIGHTS INTO THE PATHOBIOLOGY*: American Thoracic Society; 2010:A3828-A3828.
132. Patel JM, Thickett DR, Gao F, Sapey E. Statins for sepsis: distinguishing signal from the noise when designing clinical trials. *American Journal of Respiratory and Critical Care Medicine*. 2013;188(7):874.
133. Maher BM, Dhonnchu TN, Burke JP, Soo A, Wood AE, Watson RWG. Statins alter neutrophil migration by modulating cellular Rho activity—a potential mechanism for statins-mediated pleiotropic effects? *Journal of leukocyte biology*. 2009;85(1):186-193.
134. Walton GM, Stockley JA, Griffiths D, Sadhra CS, Purvis T, Sapey E. Repurposing Treatments to Enhance Innate Immunity. Can Statins Improve Neutrophil Functions and Clinical Outcomes in COPD? *J Clin Med*. 2016;5(10).
135. Gilbert R, Al-Janabi A, Tomkins-Netzer O, Lightman S. Statins as anti-inflammatory agents: A potential therapeutic role in sight-threatening non-infectious uveitis. *Porto Biomedical Journal*. 2017;2(2):33-39.
136. Sonoda K-H, Yoshimura T, Egashira K, Charo IF, Ishibashi T. Neutrophil-dominant experimental autoimmune uveitis in CC-chemokine receptor 2 knockout mice. *Acta Ophthalmologica*. 2011;89(2):e180-e188.